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Filter Paper Electrophoresis of Serum Proteins from Sharks

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VARIOUS investigators have shown that electrophoretic techniques (McDonald, 1955) are well suited for characterization of serum protein abnormalities in man (Mackay and Volwiler, 1954) and in cattle (Bradish *et al.*, 1954). However, it is difficult to find a basis of comparison of blood protein patterns found by research groups using different apparatus and procedures (Deutsch and Goodloe, 1945; Moore, 1945; and Deutsch and McShan, 1949).

These investigators used the Tiselius-type electrophoretic apparatus (Taylor, 1953) to examine sera and plasma proteins of various mammals, birds, and vertebrates. They showed that the characteristic pattern of each species is reproducible and that the differences between species, in respect to both quantity and presence or absence of components, is significant. In addition they emphasized that only patterns obtained under similar experimental conditions were comparable.

Recently, Cremer and Tiselius (1950) have shown that the components of serum protein could be separated effectively by filter paper electrophoresis. Some advantages of filter paper electrophoresis over the Tiselius methods are the smaller sample required, lower cost of equipment, and ease of use. Gleason and Friedberg (1953) used filter paper apparatus to examine the serum proteins of small animals. Drilhon (1953) examined two eels, a carp, and a dogfish. Dessauer and Fox (1956) examined about 1200 plasma protein patterns of members of the orders *Amphibia* and *Reptilia*.

In this investigation the blood serum protein patterns of four species of sharks were studied using paper electrophoretic techniques. It is hoped that further studies along these lines will be of value as a phylogenetic tool and for comparative biochemical studies of marine organisms.

Methods

Six male sharks were caught August, 1956 with a seine in the Gulf of Mexico along the shore of Galveston, Texas. The sharks were identified (Tee-van, 1948) by Mr. E. L. Arnold of this laboratory. They represented two specimens of *Scoliodon terrae-novae* or sharp-nosed shark, each 15 in. total length; two specimens of *Aprinodon isodon*, each 19 in. total length; one specimen of *Sphyrna diplana* or hammerhead shark, 18 in. total length; and one specimen of *Sphyrna tiburo* or bonnet-head shark, 19 in. total length. The fish were brought to the laboratory within one hour and appeared dead on arrival. Their hearts were exposed (all were beating) and cardiac punctures were made with glass pipets. Only one puncture was made per fish in order to avoid dilution of each blood sample with body fluids. Approximately 2 to 5 ml. of blood from each shark were placed into separate test tubes. After several hr. at room temperature (24°C–26°C), the expressed serum was removed from the clot by decanting. Serum samples were centrifuged at 8,000 rpm at 4°C. for 5 min. Aliquots of the straw-yellow sera were analyzed immediately.

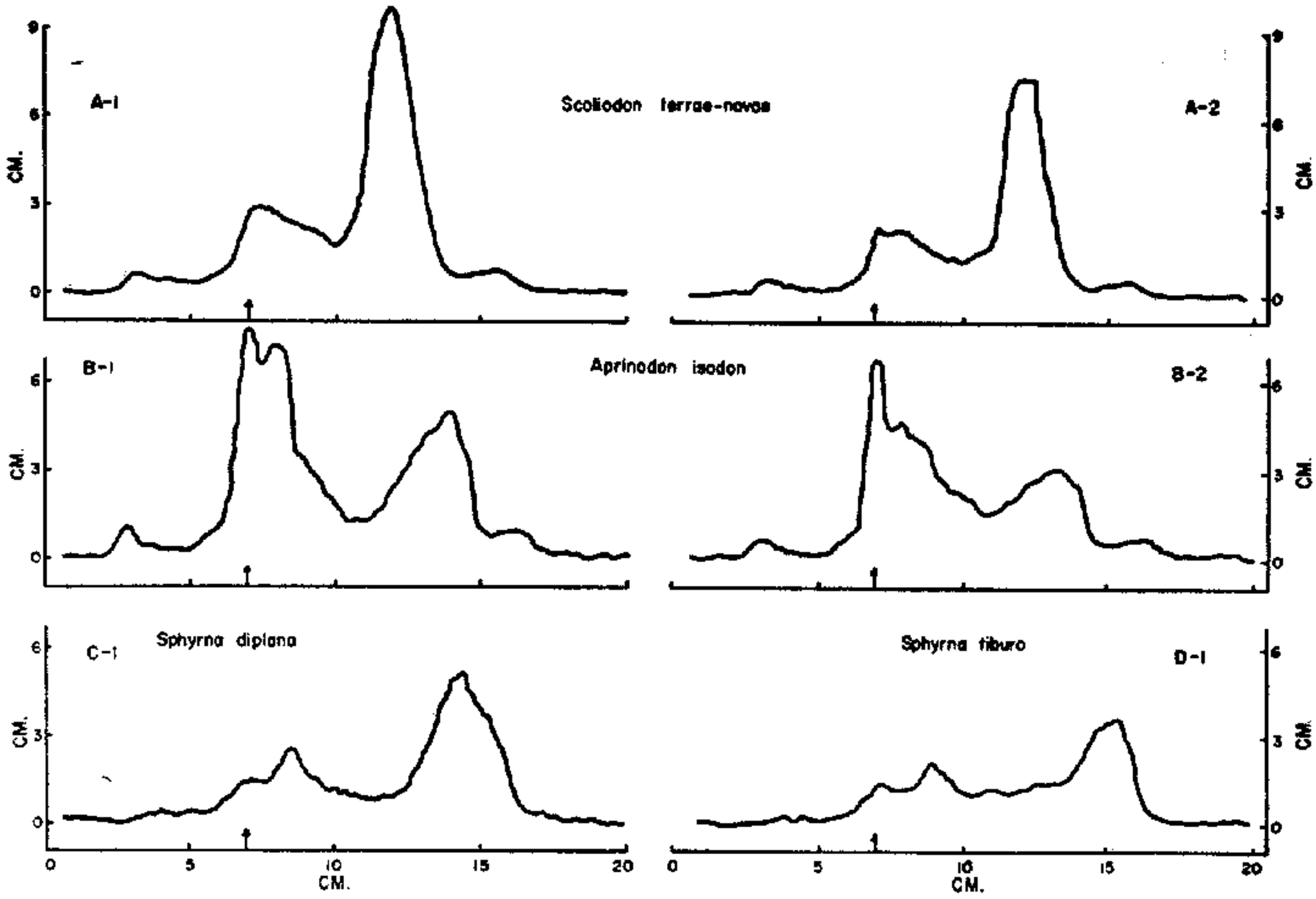

Fig. 1. Filter paper electrophoretic patterns of shark serum samples. Arrow indicates starting line.

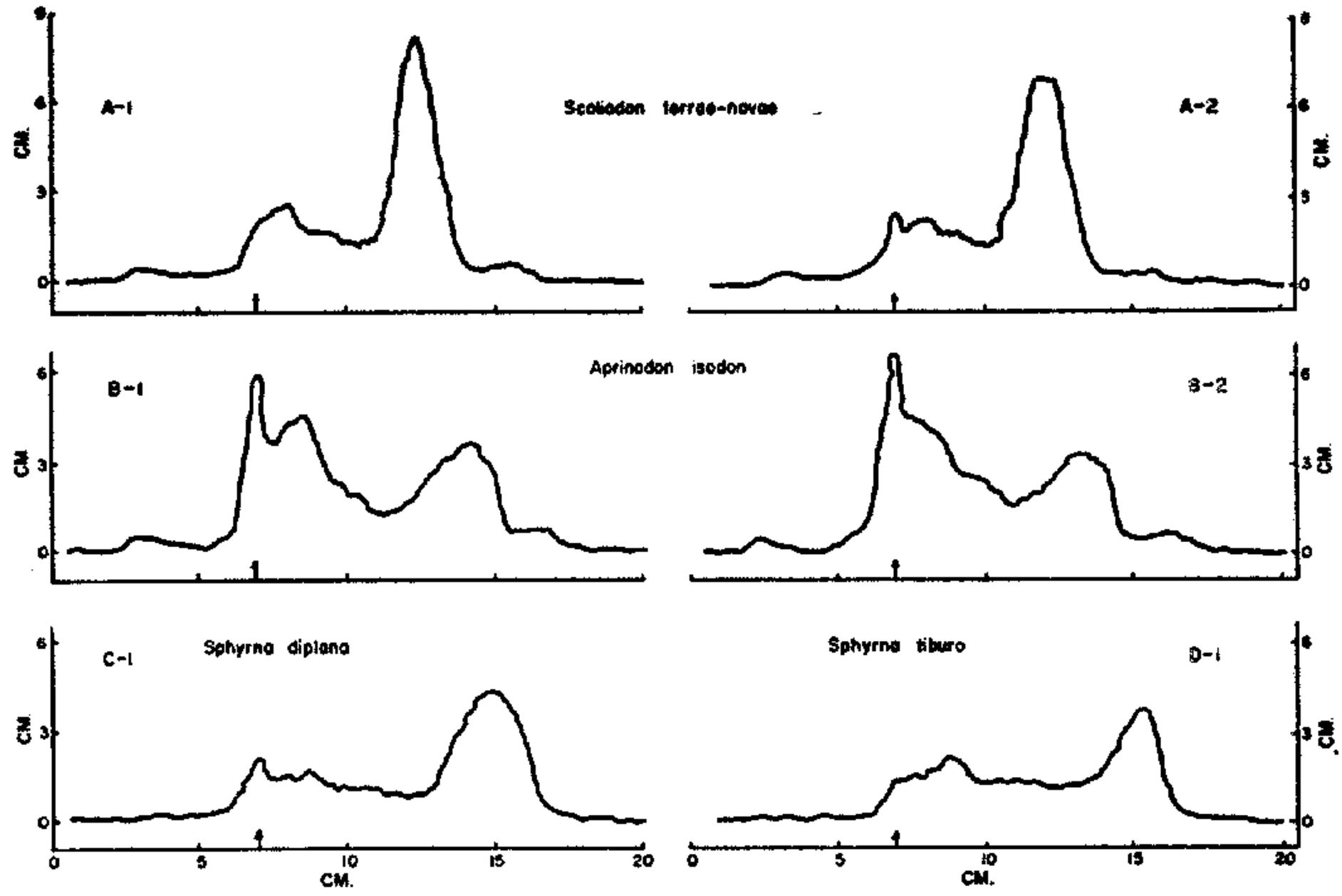

Fig. 2. Filter paper electrophoretic patterns of the same serum samples shown in Figure 1 but analyzed after one week storage in a deep freeze.

The Spinco Model R Analytrol and Duostat were used in these experiments. The general operating instructions recommended for the analysis of human sera with this equipment were followed. We used the recommended Spinco Buffer B-2 (2.76 gm. diethyl barbituric acid and 15.40 gm. sodium diethyl barbiturate per 1000 ml. distilled water) at pH 8.6 and ionic strength of 0.075. One liter of this buffer was placed into each of two electrophoresis cells with a capacity of eight paper strips each. The paper strips were saturated with buffer. After a 30 to 40 min. equilibrium period, 0.02 ml. of each undiluted sample was applied to separate paper strips with a striper which *was filled with a micropipette. A constant* current of 12 milliamperes (at 115 volts) was applied for 19 hr. at room temperature (24°C–26°C). The paper strips were then dried in an oven for 30 min. at 120°C. After immersing the dried strips in Spinco Dye B-1 (0.1 gm. bromphenol blue dissolved in 50 ml. of 95 percent ethyl alcohol plus 31 gm. zinc sulphate and made to 1000 ml. with 5 percent acetic acid) for 6 hr., the strips were rinsed twice in 1000 ml. of 5 per cent acetic acid for 6 min. periods. The paper strips were then fixed in Spinco Fixative B-2 (9.0 gm. sodium acetate dissolved in 1000 ml. of 10 per cent acetic acid) for 6 min. After blotting, the stained strips were dried in a preheated oven at 120°C for 15 min. and subsequently scanned with the analytrol.

Results and Discussion

The serum protein patterns obtained from the dyed paper strips with the analytrol are shown in Figure 1. The majority of components migrated toward the anode (right of the arrow). The sera of *Scoliodon* and *Aprinodon* contain one component which moved toward the cathode (left of the arrow). This component is hardly noticeable in samples of the two *Sphyrna* species. Patterns are similar between different individuals of the same species (A-1 and A-2, *B-1 and B-2). However, significant quantitative* differences are evident between members of the three genera as shown by the presence or absence, relative concentrations, mobility, and direction of migration of the different components. Comparison of the patterns of the two *Sphyrna* species (C-1 and D-1) shows the lesser amount of variation between different species of the same genus.

The key in Fishes of the Western North Atlantic (Tee-van, 1948) places all four genera within the suborder *Galeoidea*; the genera *Scoliodon* and *Aprinodon* are in the family *Carcharhinidae*; and the genus *Sphyrna* is in the family *Sphyrnidae*.

The same serum samples were reanalyzed (Fig. 2) after one week storage in a deep-freeze. Comparison of Figures 1 and 2 shows the minor differences that resulted and the reproducibility of the patterns by the techniques used.

The data presented in this preliminary report should be interpreted with caution. Moore (1945) studied sera patterns of rats, cats, chick and pig embryos. He showed major changes in serum patterns with age and development. He advanced the possibility that sera from the same species living under different conditions might present a pattern different from the one considered typical. Dessauer and Fox (1956) studied the plasma patterns among members of the order *Amphibia* and *Reptilia*. They found differences in patterns due to sex, age, starvation, season, and geographic variation.

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